



IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of Prof. Richard C. Willson III
and Jason Murphy

USSN 09/841,763

Filed: 04/24/2001

For: ...Methods and Compositions For Biotechnical) Examiner
Separations) Jeffrey Fredman?

Priority: ("Parent") Serial No.: 09/609,996,
Filed: 07/03/2000; and Provis. 60/143, 768,
Filed 12 July 1999,

Attorney Docket: ..009MUS

USPTO Customer Number: 26830

Response Due: **18 August 2001**

) 703 308 6568
) Fax 703 305 3014
) Art Unit 1655
) CM1-11-A13

Amendment in Response to Notice To File Corrected Application

Assistant Commissioner for Patents
Box US; Group 1743
Washington, DC 20231

Sir:

In response to the Notice To File Corrected Application mailed 06/18/01,
consideration of the following is respectfully requested.

The undersigned Attorney certifies that this Document
has been filed in the U.S. Post Office as Express Mail
ET601010102US to the USPTO on 2 July 2001 (37
CFR 1.10).

In the Drawings

Please substitute the enclosed Figures 1 – 10 (without legends) for Figures
1-10 previously filed.

In the Specification

Please amend page 32, line 19 to page 35, line 13 of the specification as
follows:

Brief Description of the Drawings

C-I-P of USSN 09/609,996

009MUSCorrectedDrawingsAmendment20010711.doc

RECEIVED

Figure 1 is a schematic diagram of preferred structures of common compaction agents

Figure 2 shows schematically the precipitation by spermidine of 40 µg/mL pBGS19luxwt or Baker's yeast RNA in 10 mM Tris buffer at pH 8.0 with and without 600 mM NaCl. (Error bars are +/- one standard deviation.)

Figure 3. Depicts a 1% agarose gel tracing the large-scale purification of pBGS19luxwt plasmid DNA. Lane 1 is a supercoiled plasmid ladder from Gibco; Lane 2 is the preparation after Celite filtration, isopropanol precipitation, and resuspension; Lane 3 is the supernatant after LiCl precipitation; Lane 4 is the supernatant of the compaction precipitation by 2.9 mM spermidine HCl; Lane 5 is the resuspended pellet of the compaction precipitation after stripping of spermidine by 300 mM NaCl, 10 mM MgCl₂, and 25 mM EDTA in 50% isopropanol; Lane 6 is a 10X loading of the material in Lane 5 (The traces of genomic DNA in these lanes can be removed by further optimization of the initial lysis and precipitation steps); Lane 7 is after a Q Sepharose anion-exchange column (See Figure 4, bottom, Peak 5); Lane 8 is a 10X loading of Lane 7 and Lane 9 is pBGSi9LUXWT plasma DNA separated using the miniprep procedure.[the same as Lane 1].

Figure 4. Shows the chromatograms from a Pharmacia FPLC System using a HP Q Sepharose anion-exchange separation of pBGS19luxwt of an alkaline lysate after isopropanol and LiCl precipitation and optional compaction precipitation. Top: NaCl gradient; Middle: with no previous

compaction precipitation step; Bottom: identical separation after a compaction precipitation step (1 volume of 2.9 mM spermidine in 10 mM Tris HCl at pH 8.0; see example 1). A Spectrum chromatography column (2.5 cm x 60 cm) packed with 150 mL Q Sepharose high performance media and equilibrated in 10 column volumes of TE with 570 mM NaCl is used. Loading and elution are performed at a linear velocity of 90 cm/hr.

Figure 5 shows schematically a summary of selective precipitation-based noncolumn DNA purification process steps for separation of DNA as disclosed in Example 1.

Figure 6. shows a 3% Biogel (from Bio101 Inc.) electrophoretic analysis of *V. proteolyticus* RNA purified by Example 9. Lane 1 is the Ambion RNA Century Plus Size Markers; Lane 2 is the lysate after BPER addition, spermidine addition, and centrifugation; Lane 3 is the supernatant of the 4 mM hexammine cobalt precipitation; and Lane 4 is the RNA pelleted in the hexammine cobalt precipitation but before any column separation.

Figure 7. shows a FPLC chromatogram of *V. proteolyticus* RNA on a 25 mL high performance Q Sepharose anion exchange column (Pharmacia). The gradient ran over 12 column volumes from 0.30 M NaCl to 0.57 M NaCl in a column buffer of 20 mM bis-tris propane and 20 mM EDTA at pH 6.9. (see Example 9)

Figure 8 shows a FPLC chromatogram of pCP3X3 aRNA-containing *E. coli* strain JM109 on a 25 mL high performance Q Sepharose anion-C-I-P of USSN 09/609.996

exchange column (Pharmacia). The gradient is run over 12 column volumes from .37 M NaCl to .57 M NaCl in a column buffer of 20 mM bis-tris propane and 20 mM EDTA at pH 6.9. (see Example 10)

Figure 9 shows a FPLC chromatogram of selective precipitation purified β ribozyme on a 25 mL high performance Q Sepharose anion exchange column (Pharmacia). The gradient is run over 12 column volumes from .37 M NaCl to .7 M NaCl in a column buffer of 10 mM bis-tris propane and 2 mM EDTA at pH 6.9. (see Example 11)

Figure 10 shows schematically a kit for convenient practice of the invention. Figure 10a shows a plasmid DNA separation kit and Figure 10b shows an RNA isolation kit, both further described under "Description of Exemplary Kits".

In The Abstract

Please substitute the enclosed shortened Abstract for that in the Application.

Remarks

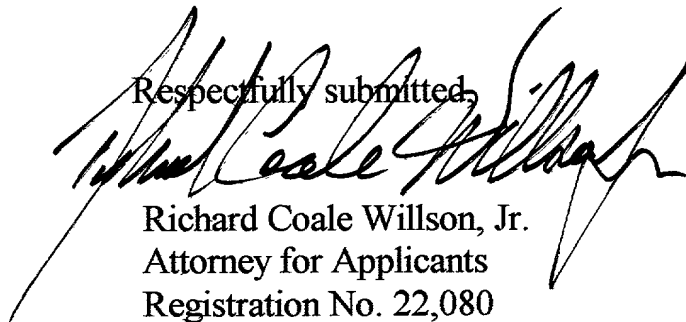
The enclosed drawings omit the detailed descriptions objected to in the Notice. The above amendments to the specification insert the substance of the omitted text into the Brief Description of the Drawings. A shortened Abstract is enclosed, without prejudice to the priority of the entire Abstract included in the application as filed. A clean copy of each of the inserts is attached hereto in compliance with the March 2001 Rules. No new matter is added. The claims are not amended.

Applicants' Attorney respectfully requests a prompt Office Action on the merits for Claims 1 – 20 herein, as licensing is being negotiated.

Any necessary (small entity) charges can be charged to USPTO Deposit Account 20-336 of Technology Licensing Co. LLC. Correspondence may be addressed to Customer No. 26830.

The Examiner is especially invited to telephone Applicants' Attorney if that would expedite prosecution and disposal of this Application.

Respectfully submitted,

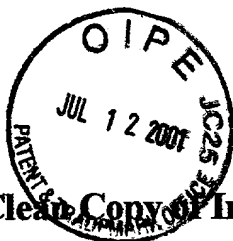


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Enclosures:

Substitute Drawings 1 – 10
Clean Copy of inserts per March 2001 Rules
Substitute Abstract (<150 words)
Copy of Notice

009MUSCorrectedDrawingsAmendment20010711.doc



Clear Copy of Insert on page 27 after line 12:

Figure 10a shows a plasmid DNA separation kit based on compaction agent precipitation technology. In the box a total of 8 solutions should be include two of which are optional. Solutions will include the 3 common alkaline lysis solutions, a low ionic strength resuspension buffer, a compaction agent precipitation solution, and a stripping solution, and optionally a 70% ethanol wash solution and a final resuspension solution.

Description of each bottle are as follows:

- A. Alkaline lysis solution I (e.g. 25 mM Tris and 10 mM EDTA at pH 8.0)
- B. Alkaline lysis solution II (e.g. 1% Sodium Dodecyl Sulfate (SDS) 0.2 N NaOH)
- C. Alkaline lysis solution III (e.g. 3 M KAc at pH 5.5)
- D. Resuspension solution (e.g. 10 mM Tris at pH 8.0)
- E. Compaction agent precipitation solution (e.g. 2 mM Spermidine 3HCl and 10 mM Tris at pH 8.0)
- F. Compaction agent stripping solution (e.g. 50% EtOH, 300 mM NaCl, 12.5 mM EDTA*)
- G. 70% EtOH wash*[#]
- H. Final Resuspension solution (e.g. High purity TE which is 10 mM Tris and 1 mM EDTA at pH 8.0).[#]

*User may have to add enough EtOH to bring the solution to the proper percentage of EtOH.

[#]Optional kit components that could be provided by end user.

Also, solutions D and E can be combined to form a resuspension/compaction agent precipitation solution.

Figure 10b shows an RNA isolation kit based on a hexammine cobalt precipitation process.

- A. Lysis/DNA precipitation solution (e.g. 50% BPER with 2.5 mM Spermidine or 1% Brij 58 with 2.5 mM spermidine in 10 mM bis tris propane at pH 7). This may also be extended for use with plant cells and other eukaryotic cells with the possibilities of homogenization,

other lysis solutions, and breaking the lysis from the spermidine DNA removal. Thus, there would be a lysis solution and a separate DNA precipitation solution.

- B. Hexamine cobalt precipitation solution (e.g. 7 mM hexamine cobalt for a total RNA precipitation or 4 mM hexamine cobalt in 10 mM bis tris propane at pH 6.9 for a high molecular weight precipitation).
- C. Optional: a second hexamine cobalt precipitation solution to bring down low molecular weight RNA not precipitated when solution B was used (e.g. 20 mM hexamine cobalt in 10 mM bis tris propane at pH 6.9).
- D. Stripping solution (e.g. 50% isopropyl alcohol* with 3 M Urea, 300 mM NaCl, 25 mM EDTA).
- E. 70% EtOH wash*[#]
- F. Final Resuspension solution (e.g. High purity TE which is 10 mM Tris and 1 mM EDTA at pH 8.0).[#]

*User may have to add enough alcohol to bring the solution to the proper percentage of alcohol.

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